Venoms to Drugs: Translating Venom Peptides into Therapeutics

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Venomous animals have a long history as a source of medical treatments (1). Snake venom, for example, has been used in Ayurvedic medicine since the 7th century BCE to prolong life and treat arthritis and gastrointestinal ailments. Tarantulas are used by indigenous populations of Central and South America to treat ailments ranging from asthma to cancer, while cobra venom has been used since the 1930s to treat conditions as diverse as asthma, polio, multiple sclerosis, rheumatism and pain (2). However, the modern era of venoms-based drug discovery did not begin until the 1970s with the development of the blockbuster antihypertensive drug captopril, based on a peptide from the venom of the Brazilian viper *Bothrops jaracaca* (3). Today, many of the major pharmaceutical companies (and most major agrochemical companies) have venom-based drug discovery programs or use venom-derived molecules for target validation (e.g., AstraZeneca, Eli Lilly, Johnson & Johnson and Merck). Moreover, there are now several companies with a focus on venom-derived therapeutics, including Airmid, ReceptoPharm (a subsidiary of Nutra Pharma), Theralpha, VenomeTech, Venomics (a subsidiary of QRxPharma) and Xenome. In this article, I outline recent developments in the venoms-based drug discovery field that might lead to new venom-derived peptide therapeutics.

Introduction

An extraordinarily diverse range of animals, including arachnids, centipedes, cone snails, reptiles, squid, and wasps, have evolved venoms for the purpose of predation (4). Since the prey–predator relationship applies a constant selection pressure on toxin efficacy, venom toxins typically have extremely high specificity and potency for their molecular target, owing to long periods of evolutionary fine tuning (>400 million years in the case of scorpions and centipedes). These features, which are often not found in natural or synthetic small molecules, have made animal toxins extremely valuable as pharmacological tools. For example, α-conotoxin peptides from marine cone snails are invaluable tools for discriminating between closely related subtypes of nicotinic acetylcholine receptors (5), while five of the seven pharmacological sites on vertebrate voltage-gated sodium (NaV) channels are defined by animal toxins (6).

The venoms of cone snails, sea anemones and arthropod predators such as centipedes, scorpions and spiders are dominated by disulfide-rich peptides that evolved via explosive duplication and diversification of a small number of ancestral toxin genes in order to produce what is essentially a combinatorial library of small, bioactive peptides (7-9). This genetic strategy led to the evolution of highly complex invertebrate venoms that can contain more than a thousand unique peptides (10,11). In contrast to invertebrate venoms, most snake venoms (with elapids being a notable exception) are less complex and contain a higher proportion of larger proteins and enzymes (12,13). Nevertheless, regardless of source species, venom proteins and peptides are usually endowed with a disulfide-rich architecture that provides them with a high degree of chemical and thermal stability as well as resistance to proteases. It is this feature, along with their selectivity and potency, that has made venoms a valuable source of lead molecules for the development of novel therapeutics.

The Current Landscape of Venom-derived Drugs

There are currently six FDA-approved drugs derived from venom peptides or proteins, with a further ten in clinical trials and many more in various stages of preclinical development (1). The majority of approved venom-derived drugs are derived from snakes or lizards and they mostly target the cardiovascular system. This implies that optimal success in venoms-based drug discovery might be achieved by narrowly focusing on cardiovascular modulators from reptiles. However, this scenario is the result of two historical factors that are no longer relevant.

First, most early toxinological studies focussed on snakes as they typically provide much larger quantities of venom than other venomous animals (typically milliliters of venom per milking compared to less than 10 µL from centipedes, scorpions and spiders). The development over the past decade of high-throughput methods for screening venoms and characterising the active components (14) has opened up the field of venoms-based drug discovery to small venomous invertebrates, which constitute the vast majority of venomous species. Second, our understanding of the cardiovascular system preceded our still rudimentary understanding of the nervous system, which houses most of the molecular targets of invertebrate venoms. Exciting neuronal drug targets such as acid-sensing ion channels (ASICs), transient receptor potential (TRP) channels, various subtypes of voltage-gated channels, and numerous G protein-coupled receptors (GPCRs) had not even been discovered in the 1970s and 1980s. It is only now that our understanding of the molecular architecture of the nervous system has become more advanced that venom peptides from invertebrates are beginning to provide therapeutic leads for non-cardiovascular targets. A good example is the introduction in 2004 of Prialt®, a voltage-gated calcium

channel blocker used for the treatment of intractable chronic pain (15). Prialt®, a 25-residue peptide stabilised by three disulfide bridges, is identical to the native peptide found in the venom of a fish-hunting marine cone snail.

Current Developments

Route of Administration of Venom-peptide Drugs

In line with an increasing industry focus on peptide drugs (1), the two most recently approved venom-derived therapeutics (Prialt® and Byetta®), as well as most venom molecules currently under development, are peptides (11–71 residues). Most are stabilised by 2–5 disulfide bonds (**Table 1**). The use of protease-resistant disulfide-rich venom peptides that can be produced by recombinant methods obviates many of the historical shortcomings of peptide drugs, such as poor stability, low solubility and high cost of manufacturing. However, since it is assumed that most venom peptides will not be orally active, these drugs are generally being developed as injectable therapies.

For life-threatening situations, perioperative use, and chronic conditions such as diabetes and multiple sclerosis, parenteral administration is unlikely to be a major limitation in terms of patient acceptance and market penetrance. However, the frequency of injection is likely to be an important factor, and consequently there is much interest in developing methods of prolonging venom-peptide half-life in order to reduce administration frequency. There has been good progress in this area as evidenced by recent developments with exenatide, the latest venom-derived drug to be approved by the FDA. Exenatide is an insulin secretagogue (GLP-1 receptor agonist) used to treat type 2 diabetes (16). It is a synthetic version of the 39-residue exendin-4 peptide isolated from the saliva of the Gila monster, a venomous lizard. Exenatide is a linear peptide with a relatively short *in vivo* half life and consequently the original formulation of this drug (Byetta®) required twice daily subcutaneous injections. Despite this limitation, Byetta® achieved worldwide sales of USD $800 million in fiscal 2009 (1). Eli Lilly, Amylin Pharmaceuticals and Alkermes subsequently developed a formulation in which the peptide is embedded in biodegradable polymeric microspheres, leading to an extended duration of exenatide release that enables therapeutic levels to be maintained with once-weekly injections (17). This new formulation (Bydureon®) was recently approved by the FDA and it has been forecast that worldwide sales will peak at USD $2.5 billion despite the introduction of competing incretin mimetics (1). This seminal work on extending the half-life of exenatide has provided both renewed impetus and a proven approach for extending the duration of action of other venom-peptide drugs.

The much smaller size of venom-peptides (typically <5 kDa) compared with larger antibody-based biologics also opens up the possibility of using non-traditional routes of administration such as buccal, nasal, pulmonary and transdermal (18). Theralpha, for example, are currently testing sublingual delivery of prohanin, an 11-residue analgesic peptide from the venom of the King cobra, while buccal and intranasal delivery are being examined for administration of the sea anemone peptide ShK (discussed in more detail below) (19). What remains to be tested in any detail is whether the small size of venom peptides and their

Table 1. Examples of disulfide-rich venom peptides currently in clinical or preclinical development.
The number of residues (size) and disulfide bridges (SS bonds) is indicated. Abbreviations: nAChR, nicotinic acetylcholine receptor; $GABA_B$, metabotropic GABA receptor; CaV2.2, N-type voltage-gated calcium channel.

Animal	Peptide	Size	SS bonds	Target	Phase	Disease
Cone snail 	Xen2714	13	2	Noradrenaline transporter	Phase IIb	Chronic pain
	Vc1.1	16	2	$GABA_B$/nAChR	Phase IIa	Chronic pain
	ω-CVID	26	3	$Ca_V2.2$	Phase IIb	Chronic pain
Sea anemone 	ShK-186	35	3	Kv1.3	Phase Ia	Autoimmune diseases
Spider 	PcTx1	40	3	ASIC1a	Preclinical	Pain/stroke
Centipede 	Ssm6a	46	3	$Na_V1.7$	Preclinical	Chronic pain

extreme resistance to proteases might confer some level of oral activity. The recent demonstration (20) that oral activity could be conferred upon a small venom peptide by increasing its stability through N-to-C-terminal cyclisation has raised hopes that oral bioavailability might prove to be a general property of disulfide-rich venom peptides.

First-in-class Drugs Derived from Venoms

One of the most exciting developments in the field in recent years has been the emergence of new, potential first-in-class drugs derived from animal venoms, best exemplified by the development of the sea anemone venom-peptide ShK for treatment of multiple sclerosis (MS) and other autoimmune diseases. In humans, expression of the voltage-gated potassium channel Kv1.3 increases ~5-fold when quiescent effector memory T (T_{EM}) cells terminally differentiate to T_{EM}-effector cells in autoimmune diseases such as MS and rheumatoid arthritis (21). In contrast, Kv1.3 expression is virtually unchanged during activation of naïve and long-lived central memory (T_{CM}) T cells. Thus, selective blockade of Kv1.3 provides a mechanism for treating T cell-mediated autoimmune diseases without inducing generalised immunosuppression (22,23). Unfortunately, although many natural and synthetic compounds have been isolated that potently inhibit Kv1.3, most are not sufficiently selective to be useful therapeutics. In contrast, ShK-186, an ShK analogue with extremely high potency *and* selectivity for Kv1.3 (21,24), recently completed a successful Phase 1a clinical trial for treatment of MS. Thus, ShK-186 might become the first-in-class Kv1.3 blocker for treatment of MS and other autoimmune diseases.

Another target for which venom peptides might provide first-in-class drugs is the voltage-gated sodium channel $Na_V1.7$, a key player in the human pain signalling pathway. Humans with inheritable loss-of-function mutations in $Na_V1.7$ are indifferent to all types of pain, with no other sensory impairments except anosmia (25). Thus, drugs that block $Na_V1.7$ should be powerful analgesics for treating many chronic pain conditions. Despite intense interest within large pharma, development of $Na_V1.7$-based analgesics has proved difficult as it is essential to avoid off-target effects on closely related Na_V channels with critical physiological roles. In particular, it is essential to avoid effects on $Na_V1.5$, which is responsible for the rising phase of the cardiac action potential, the muscle-specific $Na_V1.4$, and $Na_V1.6$, the primary Na_V channel at nodes of Ranvier. Small-molecule $Na_V1.7$ blockers tend to bind in the highly-conserved pore region of the channel, making it difficult to achieve subtype selectivity. In contrast, spider-venom peptides bind to the less well-conserved voltage sensor domains of the channel, providing an opportunity to obtain selective inhibition of $Na_V1.7$ (6). Merck have described a number of spider-venom peptides that potently inhibit $Na_V1.7$ but not with sufficient selectivity to be therapeutically useful (26). However, we recently described a centipede-venom peptide (Ssm6a) that not only potently inhibits human $Na_V1.7$ but crucially has more than 150-fold selectivity for $Na_V1.7$ over all other human Na_V subtypes with the exception of $Na_V1.2$, for which the selectivity is 32-fold (27). Ssm6a proved to be more analgesic than morphine in rodent models of pain and it appears to have a very high therapeutic index (27).

Future Prospects

Key technical advances combined with a renewed industry-wide focus on biologics have converged to provide a larger-than-ever pipeline of venom-derived peptide therapeutics. Disulfide-rich venom peptides obviate some of the potential disadvantages of therapeutic peptides and in contrast with larger biologics, they are unlikely to be immunogenic. Moreover, there is growing appreciation that oral administration might be a viable option for some venom peptides (20), significantly enhancing the likelihood of them achieving blockbuster status. The fact that some venom peptides appear capable of breaching the blood brain barrier (28) and translocating across cell membranes (29) also opens up the possibility of exploiting targets that have not previously been accessible to peptide drugs.

Despite recent progress, only a tiny fraction of the chemical diversity encoded in animal venoms has been explored. Fortunately, a much wider range of animal venoms can now be studied in detail due to recent advances in analytical techniques as well as the ability to extract peptide sequences directly from venom-gland transcriptomes (14,30). These technical advances, along with the introduction of high-throughput screening platforms (14), should greatly expedite future venoms-based drug discovery efforts and help to expand the growing pipeline of venom-derived drugs.

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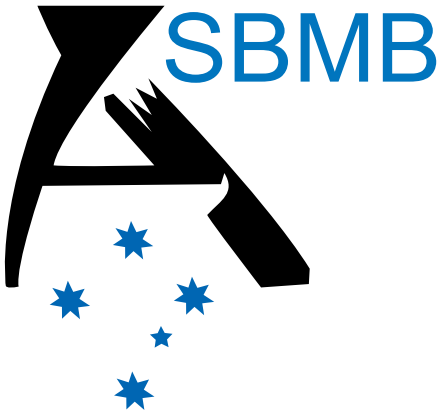

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